



# Carbon-Based Nanomaterials: Promising Antiviral Agents to Combat COVID-19 in the Microbial-Resistant Era

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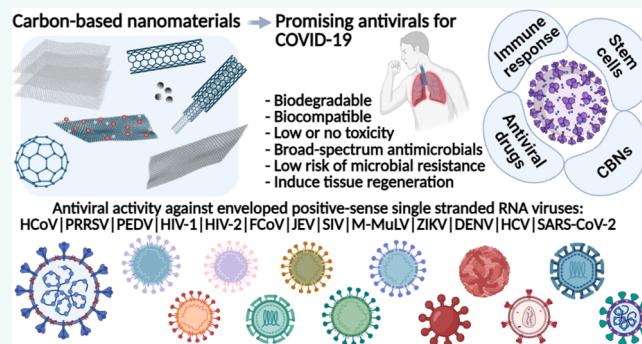
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**ABSTRACT:** Therapeutic options for the highly pathogenic human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the current pandemic coronavirus disease (COVID-19) are urgently needed. COVID-19 is associated with viral pneumonia and acute respiratory distress syndrome causing significant morbidity and mortality. The proposed treatments for COVID-19 have shown little or no effect in the clinic so far. Additionally, bacterial and fungal pathogens contribute to the SARS-CoV-2-mediated pneumonia disease complex. The antibiotic resistance in pneumonia treatment is increasing at an alarming rate. Therefore, carbon-based nanomaterials (CBNs), such as fullerene, carbon dots, graphene, and their derivatives constitute a promising alternative due to their wide-spectrum antimicrobial activity, biocompatibility, biodegradability, and capacity to induce tissue regeneration. Furthermore, the antimicrobial mode of action is mainly physical (*e.g.*, membrane distortion), characterized by a low risk of antimicrobial resistance. In this Review, we evaluated the literature on the antiviral activity and broad-spectrum antimicrobial properties of CBNs. CBNs had antiviral activity against 13 enveloped positive-sense single-stranded RNA viruses, including SARS-CoV-2. CBNs with low or no toxicity to humans are promising therapeutics against the COVID-19 pneumonia complex with other viruses, bacteria, and fungi, including those that are multidrug-resistant.

**KEYWORDS:** COVID-19, SARS-CoV-2, carbon-based nanomaterials, fullerene, carbon dots, graphene, antiviral properties, pneumonia, tissue regeneration



**H**istory has repeatedly manifested that pathogens cause disastrous effects on human beings. Thus, the recent outbreak of the recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes Coronavirus disease 2019 (COVID-19), that has spread to more than 200 countries, is a clear example. The current confirmed global COVID-19 cases and deaths have reached more than 90 million and more than 2 million, respectively.<sup>1</sup> Nevertheless, experts have suggested that many more undetected or asymptomatic cases exist,<sup>2</sup> especially in underdeveloped countries. COVID-19 continues to spread globally, threatening to collapse the health system of many developed countries such as the United Kingdom and France

that have been forced to go to a third lockdown. SARS-CoV-2 is an enveloped positive-sense, single-stranded RNA virus.<sup>3–5</sup> Its origin to this date remains enigmatic; however, multiple hypotheses have been postulated so far.<sup>6</sup> The host tropism/adaptation pattern raised questions concerning the origin of

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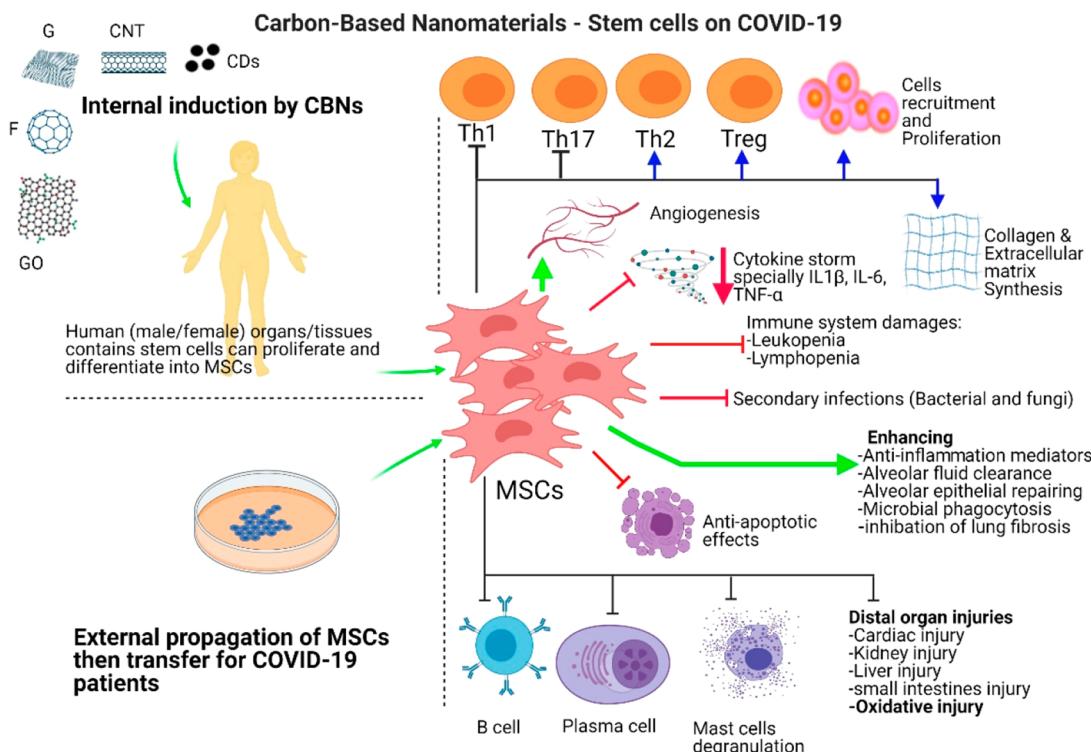
SARS-CoV-2.<sup>7</sup> SARS-CoV-2 is the seventh coronavirus known to infect humans easily<sup>8,9</sup> and only the third one causing severe pneumonia.<sup>10</sup> Viral pneumonias may be complicated by secondary microbial infections.<sup>11</sup> Thus, coinfection can be caused by viruses in the setting of community-acquired bacterial pneumonia.<sup>12–14</sup> Coinfection of COVID-19 patients is seen with the most common type of bacterial pneumonia caused by *Streptococcus pneumoniae*.<sup>15</sup> There is a great concern about the rapid spread of pathogens, such as SARS-CoV-2, that can coexist with a broad range of other types of clinically relevant microorganisms, including those which are multidrug-resistant. Therefore, the coinfection of SARS-CoV-2 with other viruses, bacteria, or fungi constitutes a real life-threatening situation to humans during the approaching cold season. In this regard, several medications have been proposed which include remdesivir, hydroxychloroquine, lopinavir/ritonavir, interferon  $\beta$ -1a, tocilizumab, favipiravir, plitidepsin, convalescent plasma infusions, and monoclonal antibodies, among many others.<sup>16</sup> However, presently, there is no effective treatment for COVID-19.<sup>17,18</sup> Furthermore, antibiotic resistance in bacterial pneumonia treatment is a widespread problem.<sup>19–21</sup> Therefore, in the quest to finding therapeutics for COVID-19, carbon-based nanomaterials (CBNs) are emerging as promising options that have shown potent antiviral activity against a broad range of enveloped positive-sense single-stranded RNA viruses,<sup>22</sup> including SARS-CoV-2, and showed low to no toxicity in humans.<sup>23–28</sup> Also, they exert an effective biocidal action against a broad spectrum of bacteria, viruses, and fungi, including multidrug-resistant strains.<sup>29–31</sup> These CBNs are mainly composed of carbon, an essential element in the human body,<sup>32</sup> and are thus biodegradable and biocompatible and can induce tissue regeneration.<sup>33–37</sup> Moreover, the development of CBNs as antiviral agents is possible, because they possess a high surface area that allows their functionalization or interaction with biocompatible polymers, which further enhances their biocompatibility and therapeutic efficacy. The epithelial response to viral challenge is well-documented to involve autophagic and apoptotic cell death.<sup>38</sup> Indeed, the primary receptor for SARS-CoV-2, the carboxypeptidase ACE-2, is a protective “survival factor” for human lung alveolar epithelial cells (AECs),<sup>39</sup> but is significantly reduced by SARS-CoV2 infection.<sup>40</sup> The cells which express the most ACE-2 in the human lung alveoli, the type II AECs, normally serve as stem cells for regeneration of lost alveolar epithelia,<sup>41</sup> but are killed by viral challenge. For these reasons, any potential therapeutic that exerts both antiviral activity and the capacity to stimulate tissue regeneration would be expected to promote lung tissue repair in the face of ongoing viral-induced cell death. Research in this area is still in its early stage, though it is predicted to grow exponentially due to the grave consequences caused by the current pandemic. The increasing number of multidrug-resistant pathogens announced by the World Health Organization constitutes another real threat to humanity in this microbial resistance era.<sup>42</sup> Therefore, there is an urgent need to find alternative antimicrobial strategies to curb the drug-resistance menace, thus providing a long-lasting treatment for the COVID-19 disease. This Review addresses the application of these broad-spectrum CBNs as antimicrobial agents by analyzing a large number of antiviral studies performed so far with CBNs against enveloped positive-sense single-stranded RNA viruses, looking at their toxicity and biodegradability and deciphering how they could defeat

microbial resistance. This vision could surpass substantially any technological paradigms that currently exist or are under development as a part of advanced therapeutics to treat COVID-19.

## NEXT GENERATION OF ANTIMICROBIALS: CARBON-BASED NANOMATERIALS

Antiviral activity of viral infection compounds can be classified into two different subgroups: (1) virus-inhibiting compounds (usually cellular); or (2) compounds that augment host defense by improving or altering virus infections (immuno-modulating agents).<sup>43</sup> Antiviral mechanisms usually require an inhibitory effect of a particular viral cell cycle essential in viral replication. As viral replication mainly depends on the host cell's metabolism as viruses are obligate intercellular parasites, valuable antivirals can disrupt viral-specific functions or at least obstruct virus-directed functions as opposed to the host cell. Therefore, the spectrum of antivirals generally is restricted. Many existing antiviral agents block host cell surface attachment followed by conformational changes of the viral capsid, leading to the uncoating of the viral genome, preventing the virus from penetrating the target cell. Others, such as nucleoside or nucleotide analogues, are antiviral drugs that provide an impairment of nucleic virus synthesis as their mode of action. An essential factor for antiviral activity is the inhibition of virus attachment to a cellular receipt or viral entry, inhibiting the viral from entering the cells. Many viruses involve proteolytic cleavage of polypeptide precursors, utilizing protease inhibitors as antivirals to active, vital viral proteins. Some of the antivirals inhibit the integration of the viral genome with the host cells' genome. Other antivirals inhibit the uncoating (viral disassembly) and therefore disturb the viral life cycle. Finally, the virus release from the host cells is inhibited through the prevention of viral clumping, thus inhibiting cell-to-cell movement of viruses through enzymatic inhibition.<sup>44–46</sup>

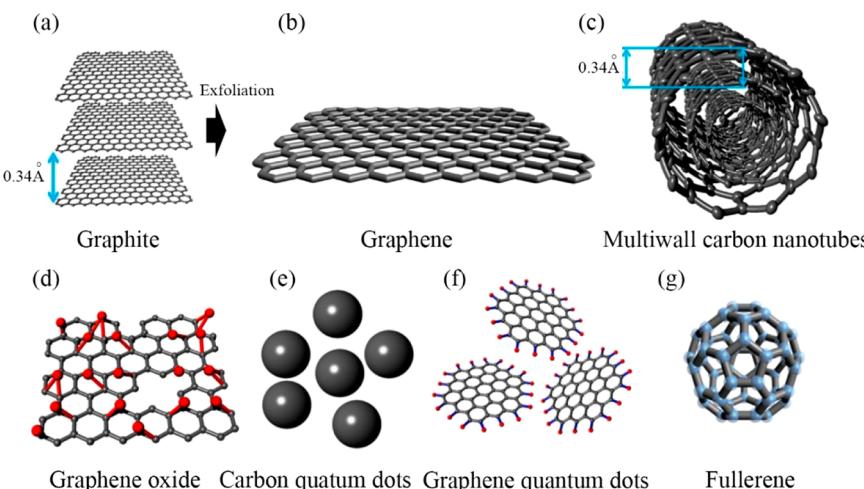
To control the ever-increasing range of infections caused by multidrug-resistant microorganisms and viruses,<sup>42</sup> the use of antibiotics or alternative antimicrobial agents such as the ionic and/or oxidized form of metals,<sup>47</sup> quaternary ammonium compounds,<sup>48</sup> peptoids,<sup>49</sup>  $\alpha$ -peptides,<sup>50</sup> and  $\beta$ -peptides<sup>51</sup> present diverse problems including drug resistance after long-term utilization. In this regard, alternative materials such as CBNs with intrinsic broad-spectrum antimicrobial activity<sup>52–63</sup> represent a promising option that would probably overcome the microbial resistance problem due to their differential antimicrobial mechanisms. Thus, the antimicrobial action of CBNs, such as graphene (G), is often attributed to a combination of several physical and chemical mechanisms: directly on the microbial particle such as (peptidoglycan membrane structure disruption, entrapment of microorganisms, transfer of electrons) and/or indirectly through induction of oxidative stress by reactive oxygen species (ROS).<sup>64,65</sup> Other nanomaterials such as those based on silver, copper, titanium, or zinc nanoparticles, for example, have shown strong broad-spectrum antimicrobial properties.<sup>47,66–69</sup> However, the existence of microbial resistance to these nanomaterials<sup>70–76</sup> and their high toxicity to mammalian cells<sup>77–81</sup> render these materials less promising for long-term therapeutics. Therefore, CBNs are increasingly proposed as the next generation of antimicrobials against multidrug-resistant infections. They possess unique properties that include very high surface area, excellent electrical and thermal conductivity, biocompatibility,



**Figure 1.** General panorama for external propagated and differentiated mesenchymal stem cells (MSCs) or internal induction of many tissues containing MSCs by CBNs (G: graphene, GO: graphene oxide, F: fullerene, CDs: carbon dots, or CNT: carbon nanotubes). MSCs have various roles in COVID-19 and/or recovered patients through secretion and modulation of physiological and immunological networks. SARS-CoV-2 infection causes many pathophysiological changes such as tissue inflammation, immune system damages (leukopenia, lymphopenia), respiratory microstructure and distal organ injury and secondary infections, and microvascular system damage. CBNs in combination with MSCs have the potential to target these pathophysiological events, acting as an alternative strategy for treating COVID-19 patients.

and the possibility to be combined with engineered polymers to develop advanced antimicrobial biomaterial composites.<sup>2829,82–85</sup> Furthermore, a recent study about the paramount concern of this proposal regarding the interaction of CBNs with the respiratory system showed that a single exposure of several CBNs (at ~0.3 and 1  $\mu\text{g}/\text{cm}^2$ ) did not manifest any adverse effects under acute exposure scenarios after 24 h.<sup>86</sup> Regarding the biodegradability of CBNs, it has been demonstrated that the human myeloperoxidase, a peroxide enzyme released by neutrophils, degraded graphene and its derivatives,<sup>87,88</sup> leading to biodegradation of graphene-based nanomaterials (GBNs) in the blood after 14 days.<sup>89</sup> Some signals of *in vivo* degradation of graphene were reported in the lungs, liver, kidneys, and spleen upon 90 days.<sup>90</sup> Oxidized forms of GBNs have shown higher susceptibility to degradation than the reduced forms.<sup>91</sup> The degradation products of graphene oxide (GO) with different dimensions exhibited no genotoxicity to human lung cells.<sup>92</sup> *In vivo* studies have shown that a concentration of 1.0 mg/kg of small GO particles (148–160 nm) tends to accumulate mainly in the liver with a lower amount in the spleen and lungs and remains longer in circulation than large particles (556–780 nm),<sup>93</sup> which were mostly present in lungs. However, when the concentration of injected GO increased 10-fold, the smaller GO particles accumulated in the lungs instead of the liver, thereby potentially increasing their efficacy for treating pulmonary infections. GO and reduced GO (rGO) of different lateral dimensions (10–800 nm) in a concentration of 1 mg/kg exhibited long blood circulation times of 14 days after

intravenous administration in mice and low uptake by the endothelial reticulum. No pathological variations were detected in the analyzed organs.<sup>85</sup> Biodegradation and cytotoxicity toward different cell lines depended on concentration, exposure time, oxidation degree, lateral size, and cell type<sup>94–97</sup> and could be modified by functionalization.<sup>94,98–101</sup> Smaller and more oxidized GBNs seem to be more cyocompatible than nonoxidized and larger particles.<sup>99</sup> Proteins, such as bovine serum albumin, adsorbed on the GBNs' surfaces, seem to have a protective effect on the hemolytic potential.<sup>102–105</sup> The biocompatibility of CBNs depends on their concentration, oxidation degree, lateral size, and dispersibility.<sup>106–114</sup> The inflammation and other effects on cells and blood components are minimal when a lower concentration of CBNs is applied. Oxidized and smaller GBNs are more biocompatible and accessible to biodegradation in the body. CBNs have shown the potential to support the growth, proliferation, and differentiation of stem cells into different tissue lineages.<sup>35,115</sup> These features potentiate the use of CBNs in combination with stem cell therapies for tissue regeneration as well as for COVID-19 patients. SARS-CoV-2 infection is accompanied by many physiopathological changes and could reach all human organs harnessing several pathways;<sup>116</sup> during this, it reaches and damages the vascular endothelial cells. CBNs can induce the production of angiogenic factors, which initiate a series of signaling pathways to induce angiogenesis through promoting the proliferation and differentiation of endothelial cells or mesenchymal stem cells (MSCs).<sup>117–119</sup> In addition to the MSCs not infected



**Figure 2.** Main carbon-based structures studied against enveloped positive-sense single-stranded RNA viruses: (a) Graphite. Reprinted in part with permission under a Creative Commons CC BY 4.0 License from ref 132. Copyright 2019 MDPI. (b) Graphene. Reprinted in part with permission from ref 131. Copyright 2019 Elsevier. (c) Multiwall carbon nanotubes. Reprinted in part with permission under a Creative Commons CC BY 4.0 License from ref 132. Copyright 2019 MDPI. (d) Graphene oxide. Reprinted in part with permission under a Creative Commons CC BY 4.0 License from ref 132. Copyright 2019 MDPI. (e) Carbon quantum dots. Reprinted in part with permission under a Creative Commons CC BY 4.0 License from ref 132. Copyright 2019 MDPI. (f) Graphene quantum dots. Reprinted in part with permission under a Creative Commons CC BY 4.0 License from ref 132. Copyright 2019 MDPI. (g) Fullerene. Reprinted in part with permission from ref 131. Copyright 2019 Elsevier.

with SARS-CoV-2, it is of note that the stem cells (specially the mesenchymal stem) represent one promising strategy for COVID-19 therapy, specifically those in moderate and/or severe infection.<sup>120–123</sup> Also, MSCs downregulate Th1 and Th17 inflammation immunity and upregulate the anti-inflammation immunity of Th2 and Treg cells, and they enhance the recruitment and proliferation of many productive cells and supportive materials (collage and extracellular matrix). Because of its potential, up to 21.02.2021, 9 (out of 91) clinical trials have been completed.<sup>124</sup> MSCs are capable of self-replication and differentiation into specialized functions to replace the disrupted and/or dysfunctioning cells/tissues.<sup>125</sup> SARS-CoV-2 induces its pathological changes *via* exacerbating cytokine production, which leads to many complications that may become severe in some cases. Stem cells have specific cytokines<sup>126</sup> that tightly derive immunomodulation (Figure 1), which may not only be helpful in controlling the SARS-CoV-2 infection severity<sup>125,127–129</sup> but also extend beyond patient recovery specifically from severe infection.<sup>130</sup>

## ANTIVIRAL PROPERTIES OF CARBON-BASED MATERIALS

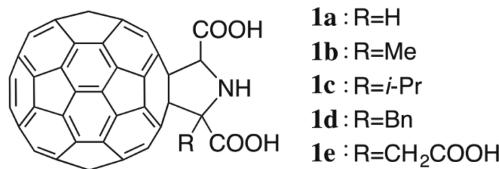
This section analyzes the antiviral properties of CBNs with different carbon-based structures (Figure 2), such as fullerene, carbon dots, graphene, and derivatives against 13 enveloped positive-sense single-stranded RNA viruses, such as SARS-CoV-2.

**Fullerene and Derivatives.** Fullerene is a zero-dimensional allotrope of CBNs with antiradical and antioxidant properties.<sup>133,134</sup> Due to the high hydrophobicity character of pristine fullerene, antiviral fullerene derivatives can be synthesized to produce hydrophilic drugs that can be easily dispersed in aqueous media.<sup>30</sup> Studies on fullerenes as antiviral agents started in 1993 on human immunodeficiency virus type 1 (HIV-1) infections.<sup>135</sup> In that study, compound 1 showed effective *in vitro* antiviral activity. Another study showed that a bis(monosuccinimide) derivative of *p,p'*-bis(2-aminoethyl)-

diphenyl-C<sub>60</sub> was actively inhibiting HIV-1 and also the type 2 (HIV-2) in acutely or chronically infected human lymphocytes and against 3'-azido-3'-deoxythymidine-resistant HIV-1. Another subsequent study showed a water-soluble fullerene-peptide conjugate capable of interacting, albeit weakly, with the HIV-1 protease.<sup>136</sup> In 1996, nine functional derivatives of C<sub>60</sub>-fullerene compounds displayed antiviral capacity at low micromolar concentrations.<sup>137</sup> Furthermore, three of these compounds exhibited antiviral activity at lower concentrations than any fullerene derivative reported to that date. In 1997, nonderivatized fullerene (buckminsterfullerene) showed *in vitro* antiviral activity against another two enveloped positive-sense single-stranded RNA viruses similar to SARS-CoV-2: the Moloney murine leukemia virus (M-MuLV) and the simian immunodeficiency virus (SIV).<sup>138</sup> However, a study performed in 2003 tested a series of fullerene derivatives (13 in total, compounds 1–13) against HIV-1 and HIV-2. The results of that study showed that some of these CBNs (6 (*trans*-2), 7 (*trans*-3), 8 (*trans*-4), 9 (*equatorial*), 12 (*trans*-2)) exhibited potent antiviral activity against HIV-1 but not HIV-2 in the low micromolar concentration range.<sup>139</sup> Nonetheless, cationic fullerene derivatives showed broader anti-HIV properties.<sup>140</sup> In 2007, chlorofullerene was developed as a precursor for the straightforward synthesis of antiviral fullerene derivatives against HIV with high solubility in water.<sup>141</sup> A later study in 2011 showed that derivatives of C<sub>70</sub>-fullerene (*i.e.*, the fullerene molecule consisting of 70 carbon atoms) exhibited high water solubility and virucidal activity against HIV and influenza virus.<sup>26</sup> A series of fullerene derivatives (Figure 3) showed potent viral inhibition of hepatitis C virus (HCV).<sup>142</sup>

Recently, tridecafullerenes appended with up to 360 1,2-mannobiose molecules showed outstanding antiviral activity against zika virus (ZIKV) and dengue virus (DENV).<sup>24</sup> Examples of antiviral studies performed with fullerenes and their derivatives are summarized in Table 1.

**Carbon Dots and Derivatives.** Carbon dots (CDs), also known as carbon quantum dots (CQDs) or Cdots, are other



**Figure 3.** Chemical structure of fullerene derivatives **1a–1e**. Reprinted with permission from ref 142. Copyright 2016 Elsevier.

members of the CBN family with small dimensions up to 10 nm in diameter that are cost-effective and environmentally inert.<sup>23,156</sup> They possess an organic molecule's chemical functionality, show a very high surface-to-volume ratio, and can be homogeneously dispersed in water. Many CDs are explored in several fields, such as chemical sensing, bioimaging, electrocatalysis, and other applications.<sup>62,157</sup> The antiviral activity of functionalized CDs produced from 4-amino-phenylboronic acid against human coronavirus (HCoV) infections has been recently demonstrated in human Huh-7 liver cells<sup>22</sup> (see Table 1). Benzoxazine-monomer-derived carbon dots (BZM-CDs) were effective against the Japanese encephalitis virus (JEV), ZIKV, and DENV.<sup>146</sup> The antiviral activity of highly biocompatible glycyrrhizic-acid-based carbon dots (Gly-CDs) synthesized from glycyrrhizic acid was demonstrated against large-enveloped RNA viruses by using the porcine epidemic diarrhea virus (PEDV) as a viral model of the coronavirus (CoVs).<sup>54</sup> This virus belongs to the *alphacoronavirus* genus, and it cannot be transmitted to humans.<sup>158</sup>

#### Graphene and Related Carbon-Based Nanomaterials.

Graphene and its oxidized form, GO, are 2D CBNs with excellent physical and biological properties<sup>159–163</sup> that can be used successfully to detect and capture viruses<sup>147,164</sup> (see Table 1). The antiviral activity of carbon nanofibers (CNFs) incorporated into alginate was reported using a nonenveloped double-stranded viral model.<sup>165</sup> However, the antiviral capacity of CNFs has never been tested against an enveloped virus belonging to the same Baltimore group<sup>166</sup> such as SARS-CoV-2. Functionalized multiwall carbon nanotubes (MWCNTs), another type of carbon-based filamentous nanomaterial, exhibited potent viral inhibition against HIV.<sup>148</sup> In this study, the effect of hydrophilicity and dispersibility of the nanomaterials showed to be the key to control the antiviral activity of MWCNT-based nanomaterials. Carboxylated MWCNT (ox-MWCNT sample) and drug-conjugated MWCNT (MWCNT-C-CHI36) showed high antiviral activity contrary to pristine MWCNT. GO has also shown potent antiviral activity with nonionic polyvinylpyrrolidone (PVP) in contrast to its combination with the cationic poly(diallyl dimethylammonium chloride) (PDDA).<sup>28</sup> The combination of silver nanoparticles (AgNPs) with GO inhibited the infectivity of enveloped feline coronavirus (FCoV) by 25% compared to 16% for GO.<sup>151</sup> AgNPs are well-known alternative antiviral agents that interact with cell surface receptors and block virus entry into the host cells.<sup>66</sup> In the same research line, GO–AgNP nanocomposites showed better viral inhibition capacity than AgNPs or GO using a PRRSV pattern on the replication of virus.<sup>152</sup> Water-soluble graphene quantum dots (GQD) synthesized from MWCNT through oxidation and exfoliation with and without conjugated antiretroviral agents exhibited efficient viral inhibition of the HIV.<sup>154</sup> Very recently, graphene derivatives with long aliphatic

chains have shown inhibition capacity against FCoV and SARS-CoV-2 replication.<sup>155</sup> Therefore, these results confirm the potential utilization of CBNs in the fight against viruses such as SARS-CoV-2.

## CARBON-BASED NANOMATERIALS AGAINST VIRUSES

This current report analyzes the antiviral potentials of CBNs against 13 viruses. The antiviral activities were detected in 40 out of 44 studies as depicted in Figure 4.

The number of studies is greater than the 22 published papers shown in Table 1, because several CBNs and/or types of viruses were studied in some publications. To perform this count of studies, only CBNs with different chemical structural forms were considered. Thus, fullerene and its derivatives showed antiviral activity in nine studies against HIV-1,<sup>26,135,137,139–141,143,144</sup> five studies against HIV-2<sup>26,139–141,143,144</sup> out of six, and against other viruses such as SIV,<sup>138</sup> M-MuLV,<sup>138</sup> HCV,<sup>142</sup> ZIKV,<sup>24</sup> and DENV.<sup>24</sup> Carbon dots and their derivatives exhibited antiviral activity against HCoV,<sup>22</sup> HIV-1,<sup>61</sup> JEV,<sup>146</sup> ZIKV,<sup>146</sup> DENV,<sup>146</sup> PEDV<sup>54</sup> and two studies against PRRSV.<sup>54,63</sup> Graphene and related CBNs have shown antiviral activity against HIV-1 in four studies out of five,<sup>147,148,154</sup> five studies against PEDV out of seven,<sup>28,152</sup> two studies against FCoV,<sup>151,155</sup> and one study against PRRSV<sup>152</sup> and SARS-CoV-2.<sup>155</sup> All these viruses are enveloped positive-sense single-stranded RNA viruses (IV Baltimore group<sup>166</sup>). Therefore, the antiviral properties of CBNs have been tested against a wide range of viruses similar to SARS-CoV-2, which suggests that CBNs are promising nanomaterials as alternative antiviral agents against this pathogen. The viruses tested against the CBNs are shown in Table 2 with all their characteristics such as name, abbreviation, genus, family, who the virus infects, disease/action, and references.

## MECHANISM OF ACTION OF CBNs AGAINST VIRAL INFECTION

The antiviral mechanisms of fullerenes, carbon dots, graphene, and related carbon nanomaterials are still not completely understood. However, some progress in understanding the mechanism of action of these promising antiviral agents has been achieved. Details of these suggested mechanisms are discussed below.

**Fullerene and Derivatives.** Antiviral fullerene derivatives can inhibit viral entry, modify its morphology and functions, and block viral replication.<sup>30</sup> Studies about the antiviral mechanism of action of C<sub>60</sub>-fullerene derivatives against viruses such as HIV-1 and HIV-2 suggest that the C<sub>60</sub> carbon sphere fits well to the active site of some HIV enzymes such as the HIV-protease.<sup>135,137,143</sup> In the same research line, a diamido diacid diphenyl fulleroid derivative (2c) was found to be an inhibitor of HIV-1 and HIV-2 protease and reverse transcriptase at low micromolar concentrations.<sup>143</sup> More recently, the series of fullerene derivatives shown in Figure 3 exhibited potential inhibition of hepatitis C virus (HCV) NS5B polymerase and HCV NS3/4 protease.<sup>142</sup> On the other hand, the mechanism of aqueous fullerene preparations of C<sub>60</sub>-fullerene combined with PVP (C<sub>60</sub>/PVP) against influenza A virus has been reported.<sup>167</sup> Influenza A virus is a negative-sense single-stranded virus that belongs to the Baltimore group V.<sup>166</sup> However, it is an enveloped RNA virus like SARS-CoV-2. The antiviral mechanism of action of the C<sub>60</sub>/PVP complexes is

**Table 1. Studies Analyzing the Antiviral Properties of Carbon-Based Nanomaterials<sup>a</sup> against 13 Enveloped Positive-Sense Single-Stranded RNA Viruses<sup>b</sup>**

Fullerene and Derivatives	Source and Manufacture	Toxicity (CC50)	Antiviral (EC50)	Tested viruses	Tested cell line/inhibition	Year	ref.
Fullerene derivatives (Compound 1)	Synthesis of bis(phenethylamino-succinate)-C <sub>60</sub>	None (compound 1)	7 $\mu$ M	HIV-1	PBMC/HIV-1 protease	1993	135
Memethanofullerene (2c)	Synthesis of diamido diacid diphenyl fulleroid derivative	Not tested	Effective at 1 mg/mL	HIV-1 and HIV-2	HIV-1 and HIV-2 protease and reverse transcriptase	1993	143
Derivatized C <sub>60</sub> Fullerene	Synthesis of bis(monosuccinimide) derivative of <i>p,p'</i> -bis(2-aminoethyl)diphenyl-C <sub>60</sub> (compound 1)	>100 $\mu$ M (compound 1)	HIV-1 10.8 $\mu$ M, HIV-2 5.5 $\mu$ M	HIV-1 and HIV-2	PBM, H9, Vero, and CEM/ HIV-1 protease	1993	144
Bioactive fullerene peptide	Synthesis of synthon 1,2-dihydro-1,2-methanofullerene [60]-61-carboxylic acid covalently linked to the $\alpha$ -amino group of the hydrophobic 4–8 sequence of peptide T	Not tested	>640 $\mu$ M (phosphonoformate)	HIV-1 not tested, HIV-2			
Functional derivatives of C <sub>60</sub> -fullerene	Synthesis of fullerene derivatives (nine active compounds)	>100 $\mu$ M (compound 1)	0.44 $\mu$ M	HIV-1 >100 $\mu$ M, HIV-2 0.003 $\mu$ M	HIV-1 protease	1994	136
Nonderivatized fullerene (buckminsterfullerene)	C <sub>60</sub> of Gold grade purity (Hoechst AG, Frankfurt, Germany)	Not tested	6 nM	HIV-1	PBMC and Vero	1996	137
F	Bis-functionalized fullerene derivatives bearing two or more solubilizing chains	Synthesis of fullerene derivatives (13 compounds tested)	4.79 $\mu$ M ( <i>trans</i> -2) 3.02 $\mu$ M ( <i>trans</i> -3) 13.2 $\mu$ M ( <i>trans</i> -4) 6.59 $\mu$ M (equatorial) 2.93 $\mu$ M (compound 3)	0.40 $\mu$ M 0.96 $\mu$ M 2.60 $\mu$ M 1.60 $\mu$ M HIV-1 0.21 $\mu$ M, HIV-2 0.2 $\mu$ M	HIV-1 and HIV-2 (effective against HIV-1, but not HIV-2)	CEM	2003
Cationic fullerene derivatives	Synthesis of a series of regioisomeric bis-fulleropyrrolidines bearing two ammonium groups (compounds 3–7)	9.04 $\mu$ M (compound 4) 12.5 $\mu$ M (compound 5)	HIV-1 0.35 $\mu$ M, HIV-2 0.70 $\mu$ M	HIV-1 and HIV-2	CEM	2005	140
Polycarboxylic fullerene derivatives using chlorofullerene as a precursor	Friedel–Crafts arylation of C <sub>60</sub> C <sub>16</sub> with methyl esters of phenylacetic and benzylmalonic acids	>63 $\mu$ M (compound 4a) 2.9 $\mu$ M (compound 7)	HIV-1 1.08 $\mu$ M, HIV-2 2.50 $\mu$ M	HIV-1 and HIV-2	CEM	2007	141
Polycarboxylic derivatives of C <sub>70</sub> -fullerene	Synthesis of C <sub>70</sub> [ <i>p</i> -C <sub>6</sub> H <sub>4</sub> (CH <sub>2</sub> ) <sub>n</sub> COOH] <sub>8</sub> ( <i>n</i> = 2, 3) starting from chlorinated [70]fullerene precursors C <sub>70</sub> C <sub>8</sub> and C <sub>70</sub> Cl <sub>10</sub>	>43 $\mu$ M (compound 2aK) >86 $\mu$ M (compound 2bK)	HIV-1 1.2 $\mu$ M, HIV-2 4.4 $\mu$ M HIV-1 1.8 $\mu$ M, HIV-2 2.3 $\mu$ M HIV-1 3.3 $\mu$ M, HIV-2 1.7 $\mu$ M	HIV-1 and HIV-2	CEM and MDCK	2011	26
Fullerene derivatives (1a, 1b, 1c, 1d, 1e)	Synthesis of proline-type fullerene derivatives	Not tested (compound cis-1a)	N5SB 0.29 $\mu$ M, NS3/4A 0.15 $\mu$ M	HCV	N5SB polymerase and HCV NS3/4A protease	2016	142

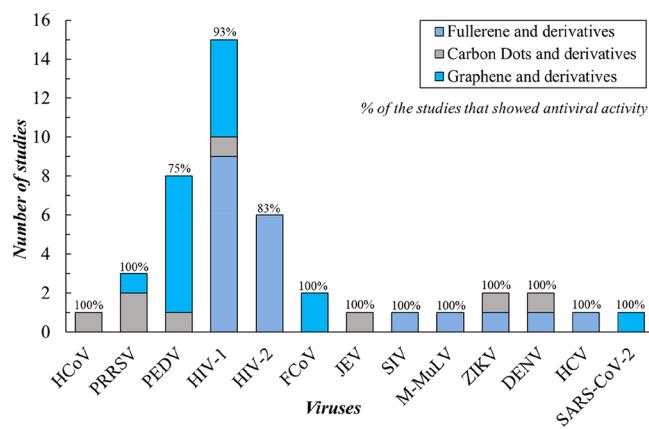
Table 1. continued

Fullerene and Derivatives	Source and Manufacture	Toxicity (CC50)	Antiviral (EC50)	Tested viruses	Tested cell line/inhibition	Year	ref.
Tridecafullerenes appended with up to 360 1,2-mannobiosides	Synthesis of multivalent disaccharide/[60]fullerene nanoballs	Not tested (compound trans-1a)	NS5B 0.23 $\mu$ M, NS3/4A 0.85 $\mu$ M	ZIKV and DENV	Jurkat	2019	24
Carbon dots and Derivatives		>10 $\mu$ M (compound 32)	ZIKV 67 pM, DENV 35 pM				
Carbon dots	Synthesis from PEG-diamine and ascorbic acid by a process of grounding, autoclaving, and purification	Toxicity (CC50)	Antiviral (EC50)	Tested viruses	Tested cell line/inhibition	Year	ref.
Boronic-acid-attributed carbon quantum dots	Synthesis by calcination of citric acid anhydrous and reaction with 4-carboxy-3-chlorobenzeneboronic acid, 4-dimethylaminopyridine, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide	>0.250 mg/mL	effective at 0.125 ng/mL only	PRRSV	PK-15 and MARC-145	2016	63
Functional carbon quantum dots	Synthesis of CQDs-5 and CQDs-6 according to the authors' protocol <sup>145</sup>	>600 $\mu$ g/mL	4.69–9.37 $\mu$ g/mL	HIV-1	MT-4/HIV-1 and MOLT-4	2016	61
Benzoxazine-monomer carbon dots	Synthesis from benzoxazine monomers and NaOH by an process of autoclaving and purification	>100 $\mu$ g/mL (CQDs-5)	10–20 $\mu$ g/mL	HCov	Huh-7	2019	22
Glycyrrhetic-acid-based carbon dots	Synthesis from glycyrrhetic acid of Chinese herbal medicine by a hydrothermal method	>100 $\mu$ g/mL (CQDs-6)	2–5 $\mu$ g/mL				
Graphene and Derivatives	Single-layered graphene modeled with the Avogadro software	>75 $\mu$ g/mL	JEV 18.63 $\mu$ g/mL, ZIKV 3.715 $\mu$ g/mL, DENV 37.49 $\mu$ g/mL	JEV, ZIKV, and DENV	BHK-21 (for JEV) and Vero (for ZIKV and DENV)	2019	146
Graphene		>0.90 mg/mL	0.30 mg/mL	PEDV, PRRSV	Vero (for PEDV), MARC-145 (for PRRSV)	2020	54
Multivalved carbon nanotubes: pristine MWCNT ( <i>p</i> -MWCNT), ox-MWCNT prepared by catalytic chemical vapor deposition from isobutane on an Fe/Al <sub>2</sub> O <sub>3</sub> catalyst and purified (>95%), ox-MWCNT with nitric acid/sulfuric acid (1:3) MWCNT; CHI360, CHI415		Toxicity (CC50)	Antiviral (EC50)	Tested viruses	Tested cell line/inhibition	Year	ref.
G		Not tested	Not tested	HIV-1	Essential target Proteins (HIVVP, Nef, and Gag)	2014	147
Graphene-based materials (GO, rGO, GO/PVP, GO/PDDA, Gt, GtO)	GO from Nanjing XFNANO Materials Tech. Gt from Sigma-Aldrich. GtO from Gt via the modified Hummers method. Prepared GO-PDDA, GO-PVP, <sup>149</sup> and rGO. <sup>150</sup>	21.22 $\mu$ g/mL (p-MWCNT)	>69.35 $\mu$ g/mL	HIV-1	MT-4	2015	148
Graphene oxide with silver nanoparticles	GO <i>via</i> Hummers' method and dispersion in a solution with AgNO <sub>3</sub> and ethylene glycol in microwave oven	11.43 $\mu$ g/mL (ox-MWCNT) 5.48 $\mu$ g/mL (CHI360) 75.73 $\mu$ g/mL (CHI415)	9.04 $\mu$ g/mL				
Graphene oxide with silver nanoparticles	GO from Chengdu Organic Chemicals and AgNO <sub>3</sub> from Sigma-Aldrich were self-assembled <i>via</i> interfacial electrostatic force	>50 $\mu$ g/mL	PRV effective at 6 mg/mL PEDV effective at 1.5 mg/mL	PRV, PEDV	Vero	2015	28
		No (GO/PDDA and Gt)	FCoV effective at 0.1 mg/mL	IBDV	fwf-4	2016	151
		PDRS effective at 0.0625 mg/mL	PRRSV effective at 4.0 $\mu$ g/mL	PDRS, PEDV	MARC-145(for PRRSV) and Vero (for PEDV)	2018	152

Table 1. continued

Graphene and Derivatives	Toxicity (CC50)	Antiviral (EC50)	Tested viruses	Tested cell line/inhibition	Year	ref.
Water-soluble GQD and drug-conjugated GQD	Water-soluble GQD prepared by prolonged acidic oxidation of <i>p</i> -MWCNT. <sup>153</sup> The anchorage of the antiretroviral drugs to the GQD surface by coupling reactions between the nucleophilic amino groups of the drugs and the carboxylic functionalities of the GQD	62.82 µg/mL (CHI499) 2.71 µg/mL (CDF119) 23.9 µg/mL (GQD-CHI499)	PEDV effective at 2.0 µg/mL 0.12 µg/mL 0.64 µg/mL 0.066 µg/mL	HIV-1 MT-4	2018	154
Graphene platforms with precise dual sulfate/alkyl functionalities	The surface of graphene is functionalized with polyglycerol sulfate (PGS) and aliphatic chains of different length (C6, C9, C10, C11, C12)	>1000 µg/mL (G-PGS-C9) 63.4 µg/mL (G-PGS-C10) 68.9 µg/mL (G-PGS-C11) 100.19 µg/mL (G-PGS-C12)	FCoV 749.4 µM, SARS-CoV-2 339.7 µM FCoV 9.8 µM, SARS-CoV-2 29.1 µM FCoV 6.3 µM, SARS-CoV-2 0.8 µM FCoV 85.2 µM, SARS-CoV-2 22.9 µM	FCoV, SARS-CoV-2 A549, HBE, Vero	155	

<sup>a</sup>Fullerene, carbon dots, graphene, and derivatives. <sup>b</sup>Source and manufacture of the CBNs, 50% cytotoxic concentration (CC50), tested viruses, tested cell line/inhibition, year, and references are indicated for each study.

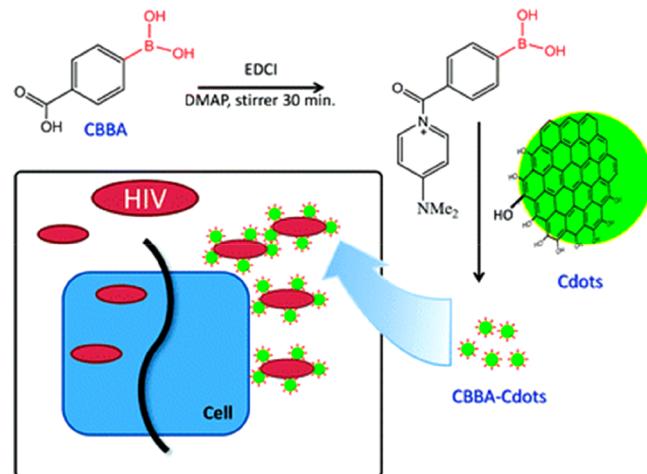


**Figure 4.** Studies of carbon-based nanomaterials' antiviral activity indicating the percentage of studies that showed antiviral activity against 13 enveloped positive-sense single-stranded RNA viruses. The carbon-based nanomaterials were in the form of fullerenes, carbon dots, graphene, and derivatives as shown in Table 1.

attributed mainly to the lipid component of virus membranes as a membranotropic agent.<sup>167</sup> Thus, the round and oval morphology of the viral particles before being in contact with the C<sub>60</sub>/PVP preparation was observed by transmission electron microscopy. The well-defined surface glycoproteins in the form of "brush" lost their integrity of viral envelopes when they were in contact with the C<sub>60</sub>/PVP complexes by breaking the lipoprotein envelope structure, and these complexes fused with the virion-like aggregates.

**Carbon Dots and Derivatives.** In the context of antiviral mechanisms, CDs could inhibit viral replication by activating the interferon response for the porcine reproductive and respiratory syndrome virus (PRRSV).<sup>63</sup> Furthermore, CDs conjugated with carboxyl phenylboronic acid (CBBA)

prevented the entry of HIV-1 viruses into cells by suppressing syncytium (Figure 5).<sup>61</sup>



**Figure 5.** Schematic illustration of conjugating carboxyl phenylboronic acid (CBBA) on Cdots (CBBA–Cdots) and different mechanisms of inhibition entry. Reprinted with permission from ref 61. Copyright 2016 Royal Society of Chemistry.

The antiviral studies of functionalized CDs produced from 4-aminophenylboronic acid against human coronavirus (HCoV) infections in human Huh-7 liver cells showed inhibition of the viral entry and effects at the replication steps, which were ascribed to the interaction of the CBNs' functional groups with the viral entry receptor DPP4<sup>22</sup> (see Figure 6).

**Graphene and Related Carbon-Based Nanomaterials.** Graphene and GO can destroy the virus surface proteins and extract their RNA by bioreduction.<sup>164</sup> The high binding affinity of graphene to the essential target proteins HIV Vpr, Nef, and

**Table 2. Information of the Enveloped Viruses Tested to Study the Antiviral Properties of CBNs Belonging to the Same Baltimore Classification of SARS-CoV-2<sup>a</sup>**

Virus name	Abbreviation	Genus	Family	Infects	Disease/action	References
Human coronavirus	HCoV	Alphacoronavirus	Coronaviridae	Humans	Common cold, pneumonia, and bronchiolitis	22
Porcine reproductive and respiratory syndrome virus	PRRSV	Betaarterivirus	Arteriviridae	Pigs	Porcine reproductive and respiratory syndrome	54, 63, 152
Human immunodeficiency virus type 1	HIV-1	Lentivirus	Retroviridae	Humans	AIDS	26, 61, 135, 139–141, 147, 148, 154
Human immunodeficiency virus type 2	HIV-2	Lentivirus	Retroviridae	Humans	AIDS	26, 139–141
Feline coronavirus	FCoV	Alphacoronavirus	Coronaviridae	Cats	Feline infectious peritonitis	151, 155
Japanese encephalitis virus	JEV	Flavivirus	Flaviviridae	Humans through Culex mosquitoes	Inflammation of the brain occurs	146
Simian immunodeficiency virus	SIV	Lentivirus	Retroviridae	Nonhuman primates	Simian AIDS	138
Moloney murine leukemia virus	M-MuLV	Gammaretrovirus	Retroviridae	Mouse	Cancer	138
Zika virus	ZIKV	Flavivirus	Flaviviridae	Humans through Aedes mosquitoes	Zika fever	24, 146
Dengue virus	DENV	Flavivirus	Flaviviridae	Humans through Aedes mosquitoes	Dengue fever	24, 146
Hepatitis C virus	HCV	Hepacivirus	Flaviviridae	Humans	Hepatitis C	142
Severe acute respiratory syndrome coronavirus 2	SARS-CoV-2	Betacoronavirus	Coronaviridae	Humans	COVID-19	155

<sup>a</sup>Group IV ((+)ssRNA<sup>166</sup>): single-stranded positive-sense RNA virus.

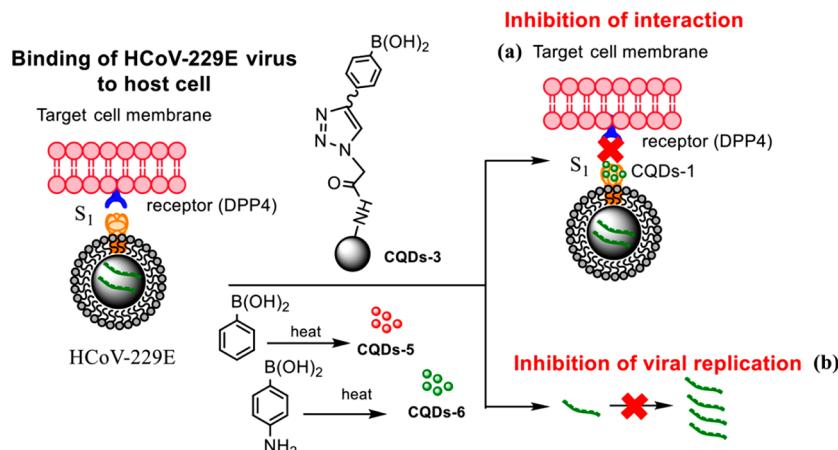


Figure 6. Influence of carbon quantum dots (CQDs) on the binding of HCoV229E virus to cells: (a) inhibition of protein S receptor interaction; (b) inhibition of viral replication. Reprinted with permission from ref 22. Copyright 2019 American Chemical Society.

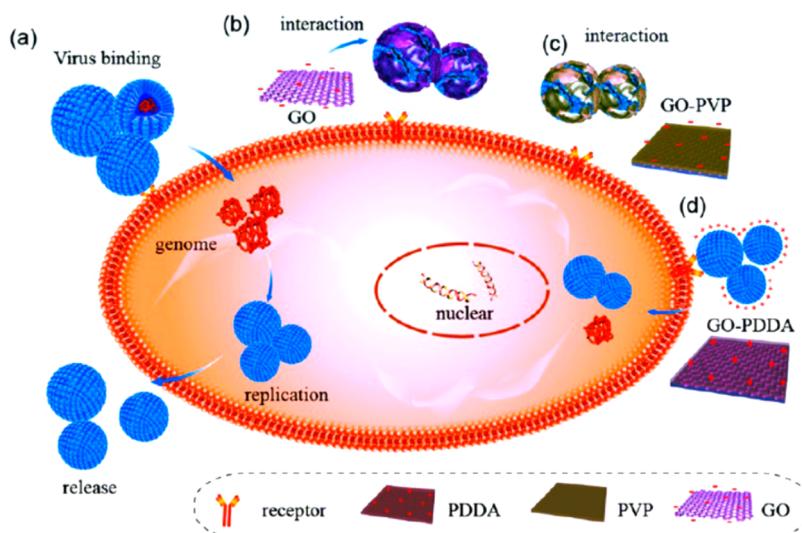


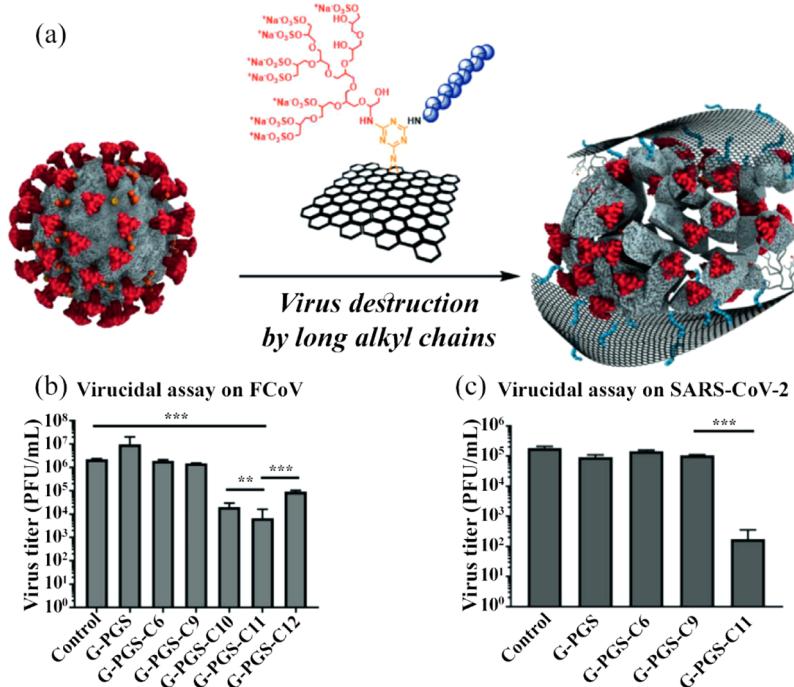
Figure 7. Possible antiviral mechanisms of graphene oxide (GO): (a) Infection initiation: virus binding by interaction with cell receptors. (b) Interaction of negatively charged GO nanosheet with the positively charged viruses, producing virus damage and infection inhibition. (c) GO conjugated with nonionic PVP blocked infection, (d) but GO with cationic PDDA did not. Reprinted with permission from ref 28. Copyright 2015 American Chemical Society.

Gag during HIV infections was reported in 2014.<sup>147</sup> Evaluation of the antiviral capacity of GO and rGO against PEDV showed significant viral inhibition by inactivating the virus before entering the cell (Figure 7).<sup>28</sup>

It was reported that the sharp edges of the GO or rGO nanosheet inactivated the virus by physical disruption of its biological structure through direct interaction, which resulted in the outflow of intracellular metabolites.<sup>28</sup> Since rGO and GO exhibited a similar viral inhibition capacity in that study, the surface functional groups present in these CBNs may play a minor role. The antiviral activity was concentration- and time-dependent incubation. Therefore, the potent antiviral activity of both GO and rGO can be attributed to the negative charge, which favors the electrostatic interaction with the positive charge of the virus and the single-nanosheet-layer structure.<sup>30</sup> On the other hand, graphite (Gt) showed no viral inhibition, and graphite oxide (GtO) exhibited weaker viral inhibition than monolayer GO and rGO, suggesting that the nanosheet form plays a significant role in the antiviral activity.<sup>28</sup> In the same research line, GO–AgNP nanocomposites prevented

PRRSV from entering the host cells (~59.2% inhibition) and improved the production of interferon- $\alpha$  (IFN- $\alpha$ ) and ISGs, which directly block the proliferation of PRRSV.<sup>152</sup> Furthermore, graphene sheets with defined dual sulfate/alkyl functionalities have shown potent antiviral activity against FCoV and SARS-CoV-2.<sup>155</sup> Here, graphene acted as a 2D platform to allow the interaction of the negatively charged polyglycerol sulfate (PGS) branches with the positively charged patches of the virus particles, and then, the long aliphatic chains (C11) ruptured the membrane of the lipid envelope. Thus, G-PGS-C11 displayed the strongest antiviral activity against SARS-CoV-2 without exhibiting significant toxicity against eukaryotic host cells (Figure 8).

Carbon-based nanomaterials may exercise multiple mechanisms against positive-sense single-stranded RNA viruses according to their physical configuration, chemical modifications, and metabolism. Hence, CBNs could work directly against the virus particle by distorting the envelope or the capsid organization; additionally, they may exert a steric hindrance effect by physically occupying a catalytic site of an



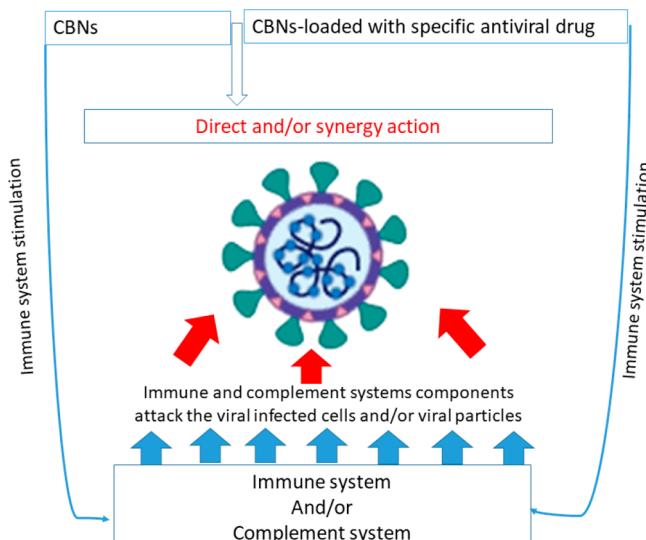
**Figure 8.** Schematic representation of virus rupturing by G-PGS-C11 (a). Virucidal assays for the functionalized graphene platforms against FCoV (b) and SARS-CoV-2 (c). Values are expressed as mean  $\pm$  standard deviation ( $n = 4$ ). Adapted with permission under a Creative Commons CC BY 4.0 License from ref 155. Copyright 2021 Wiley-VCH GmbH.

essential viral enzyme or a receptor cavity.<sup>136</sup> When chemically modified with charged residues or metabolically activated, CBNs could perform both a direct disrupting effect on the virion structure and an indirect antiviral activity due to tuning of the redox signaling and the homeostatic innate/inflammatory response of the target cells.<sup>30</sup> All of the above functions, coupled with a negligible toxicity, may account for the anti-SARS-CoV-2 therapeutic potential of carbon compounds.<sup>155</sup> An even synergistically increased inhibitory effect may be played by CBNs when loaded with specific antiviral drugs.<sup>30</sup> Thus, the direct and intrinsic antiviral activities of the compounds plus the loaded antiviral drug will work synergistically against the viral particles, which may increase the viral destruction potential.<sup>168</sup> Another characteristic feature of the CBNs is based on the immunostimulatory potentials,<sup>169,170</sup> which will derive and propagate the antiviral-cell-mediated immunity, which also works synergistically with the CBN–drugs leading to an increase in the virally infected cells and/or viral particle accessibility for CBNs or CBNs–drug achieving a more efficient viral infection clearance (Figure 9) through many suggested scenarios.<sup>171</sup>

The most versatile interesting feature of CBNs is selecting one or more entities of them and functionalizing the selection base on the target function. The planning interactions between empty or loaded CBNs and the immune system, including the complement system, can be reciprocal in the context of which immune system components would involve.<sup>171,172</sup>

## TOXICOLOGICAL ASPECTS OF CARBON-BASED NANOMATERIALS

There are widespread concerns on the toxicological aspects of CBNs, because some studies have reported that depending on the type of CBN, dimensions, oxidation degree, functionalization, concentration, and exposure time, CBNs may exert



**Figure 9.** Suggested mechanism of action of CBNs against viral infection. The CBNs could work against viral and secondary infection in three scenarios: (1) CBNs alone; (2) in synergy with loaded antiviral drugs; (3) and/or in synergy with the immune system components depending on the CBN's immunostimulatory potentials.

cytotoxicity effects on host cells.<sup>94–101</sup> Nevertheless, we focused our attention on the toxicological studies performed with the CBNs tested against the 13 enveloped single-stranded positive-sense RNA viruses analyzed in this Review (Table 1). Details of these studies are discussed below.

**Fullerene and Derivatives.** An anti-HIV-1 fullerene derivative (compound 1) showed no cytotoxicity on human PBM cells in 1993.<sup>135</sup> Another study reported noncytotoxicity for concentrations up to 100  $\mu$ M of an anti-HIV bis-

(monosuccinimide) derivative of *p,p'*-bis(2-aminoethyl)-diphenyl-C<sub>60</sub> in peripheral blood mononuclear (PBM) cells and H9 human embryonic stem cells, Vero, and CEM cells.<sup>144</sup> In 1996, nine anti-HIV functional derivatives of C<sub>60</sub>-fullerene showed no cytotoxicity for concentrations also up to 100 μM. However, a series of anti-HIV-1 bis-functionalized fullerene derivatives were developed after that in 2003, and only one of them (derivative 1) exhibited moderate toxicity.<sup>139</sup> Nonetheless, low cytotoxic properties were reported for anti-HIV cationic fullerene derivatives and anti-HIV water-soluble fullerene derivatives in 2005 and 2007, respectively.<sup>140,141</sup> A later study in 2011 showed that anti-HIV derivatives of C<sub>70</sub>-fullerene also exhibited low toxicity *in vitro* and *in vivo*.<sup>26</sup> More recently, an anti-HCV fullerene derivative (derivative 1a, Figure 3) showed no cytotoxicity in the low micromolar range.<sup>25</sup> Furthermore, very recently, anti-ZIKV and anti-DENV tridecafullerenes appended with up to 360 1,2-mannobiose molecules showed no cytotoxicity in the picomolar range.<sup>24</sup>

**Carbon Dots and Derivatives.** CDs are highly promising nanomedicine tools for antiviral applications due to their low toxicity and potent antiviral activity.<sup>157</sup> Thus, CDs showed antiviral activity against PRRSV and low cytotoxicity in MARC-145 cells.<sup>63</sup> Furthermore, the modified CDs or their combination with other compounds significantly decreased the cytotoxicity of these carbon-based antiviral nanomaterials.<sup>22,54,61,146</sup> For example, in the cytotoxicity tests of both Cdots and CBBA–Cdots at high concentrations (up to 300 mg·mL<sup>-1</sup>), the proliferation of human cells was as fast as that of the untreated cells (control) after 24 h of incubation, suggesting the absence of cytotoxic effects for both types of nanomaterials.<sup>61</sup> However, this study did not assess ROS or cytokine generation. Immunomodulatory effects are known to be induced by other carbon-based nanoparticles such as graphene (see the next paragraph). Anti-HCoV CDs produced from 4-aminophenylboronic acid, BZM-CDs with anti-JEV, anti-ZIKV, and anti-DENV properties, and anti-PEDV and anti-PRRSV Gly–CDs manifested no cytotoxicity.<sup>22,54,146</sup>

**Graphene and Related Carbon-Based Nanomaterials.** The toxicological analysis of pristine MWCNT, ox-MWCNT, and MWCNT–C-CHI36 with anti-HIV-1 properties showed opposite results.<sup>148</sup> Thus, ox-MWCNT and MWCNT–C-CHI36 showed low cytotoxicity contrary to pristine MWCNT, which exhibited high cytotoxicity. However, GO showed potent anti-PEDV using a noncytotoxic concentration ( $\leq 6 \mu\text{g}/\text{mL}$ ).<sup>28</sup> Anti-FCoV GO–AgNP nanocomposites and neat GO could also be prepared at very low or noncytotoxic concentrations.<sup>151</sup> In the same way, anti-PRRSV GO–AgNP nanocomposites could be prepared at a noncytotoxic concentration ( $\leq 4 \mu\text{g}/\text{mL}$ ).<sup>152</sup> Finally, a water-soluble GQD with a conjugated antiretroviral agent (GQD–CHI499) was selected as an excellent potential candidate due to its high antiviral activity against HIV and low cytotoxicity.<sup>154</sup> Anti-FCoV and anti-SARS-CoV-2 graphene platforms have shown a large concentration window without significant toxicity against human cells.<sup>155</sup> Therefore, the lack of toxicity could provide great potential in the development of safe therapeutics based on carbon-based nanomaterials to combat COVID-19 in the microbial-resistant era.

## CONCLUSIONS

Carbon-based nanomaterials have been evaluated for their antiviral activity against 13 enveloped viruses (HCoV, PRRSV, PEDV, HIV-1, HIV-2, FCoV, JEV, SIV, M-MuLV, ZIKV,

DENV, HCV, and SARS-CoV-2), all single-stranded positive-sense RNA viruses belonging to the Baltimore group IV. Most of the studies have shown a potent antiviral activity and from low to no toxicity, supporting the potential for the use of CBNs to combat SARS-CoV-2. As a revolutionary technology approach to treat COVID-19, these carbon-based therapeutics can provide a significant breakthrough, as these nanomaterials allow the targeting of microbial resistance issues and can potentially induce tissue regeneration at the same time. Furthermore, these antimicrobial nanoweapons could be employed to deal with SARS-CoV-2 alone or with other types of viruses, bacteria, or fungi causing pneumonia, including multidrug-resistant strains. The chance of success in applying these wide-spectrum antimicrobial nanomaterials is very high because of the preliminary antiviral results reported for 13 viruses and the fact that the proposed approach could be extended to other types of pneumonia caused by other important pathogens.

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Á.S.A. conceived the idea of this work, wrote the draft manuscript, and performed major editing. K.T., A.T.M., M.S., V.N.U., K.L., P.A., A.A.A.A., G.C., R.K., A.L., B.D.U., D.B., E.M.R., N.G.B., and Y.K.M. edited the manuscript, and S.S.H., P.P.C., G.P., M.M.T., T.M.A.E.A., S.S., and A.M.B. proofread the manuscript.

## Notes

The authors declare no competing financial interest.

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## VOCABULARY

**coronavirus disease 2019 (COVID-19)**, viral infectious disease that has spread throughout the world causing the current COVID-19 pandemic; **acute respiratory syndrome coronavirus 2 (SARS-CoV-2)**, is the causative pathogen responsible for the COVID-19 disease; **carbon-based nano-materials (CBNs)**, are materials mainly constituted by carbon

atoms that possess a broad range of antimicrobial properties; **enveloped positive-sense single-stranded RNA viruses**, are viruses that belong to the IV Baltimore group similar to SARS-CoV-2; **pneumonia**, is an infection of the lungs caused by bacteria, viruses, or fungi.

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